Generalization of fear to respiratory sensations

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Abstract

Interoceptive fear conditioning (IFC), fear generalization and a lack of safety learning have all been hypothesized to play a role in the pathogenesis of panic disorder, but have never been examined in a single paradigm. The present study aims to investigate whether healthy participants (N=43) can learn both fear and safety to an interoceptive sensation, and whether such learning generalizes to other, similar sensations. Two intensities of inspiratory breathing impairment (induced by two pressure threshold loads of 6 and 25 cmH_20) served as interoceptive conditional stimuli (CSs) in a differential conditioning paradigm. An inspiratory occlusion was used as the unconditioned stimulus (US). Generalization was tested 24h after conditioning, using four generalization stimuli with intensities in-between CS+ and CS- (GSs: 8-10.5-14-18.5 cmH_20). Measures included US-expectancy, startle blink EMG responses, electrodermal activity and respiration. Perceptual discrimination of interoceptive CSs and GSs was explored with a discrimination task prior to acquisition and after generalization. Results indicate that differential fear learning was established for US-expectancy ratings. The group with a low intensity CS+ and a high intensity CS- showed the typical pattern of differential fear responding and a similarity-based generalization gradient. In contrast, the high intensity CS+ and low intensity CS- group showed impaired differential learning and complete generalization of fear. Our findings suggest that interoceptive fear learning and generalization are modulated by stimulus intensity and that the occurrence of discriminatory learning is closely related to fear generalization.

Key words: Interoception, fear conditioning, generalization, dyspnea, discrimination, panic disorder

Introduction

Human fear conditioning is extensively used as an experimental model to study and understand the etiology and maintenance of panic- and anxiety disorders (Bouton, Mineka, & Barlow, 2001; Mineka & Zinbarg, 2006). In fear conditioning, an initially neutral conditioned stimulus (CS) is paired with an intrinsically aversive stimulus (unconditioned stimulus, US) and through associative learning the CS-US pairing results in the CS becoming a predictive signal for imminent threat, typically eliciting conditioned fear responses (CR). These conditioned fear responses may consist of physiological arousal, subjective apprehension or verbal responses and avoidance behavior (Lang, 1971). In differential fear conditioning paradigms, two CSs are used of which one is paired with an aversive US (CS+: learned danger cue) and the other is presented in the absence of the US (CS-: learned safety cue), resulting in larger fear responses to the CS+ versus the CS-. Pathological forms of fear learning are considered hallmarks of psychopathologies, including anxiety disorders, depression and post-traumatic stress disorder (Pollak et al., 2008). For instance, a meta-analysis across 453 anxiety patients and 455 healthy comparisons suggested enhanced fear-responding to the CS- instead of elevated differential responding to the CS+ as one of the robust conditioning correlates of clinical anxiety (Lissek et al., 2005). The enhanced fear-responding to the CS- may imply enhanced generalization of conditioned fear from the danger cue (CS+) to the resembling safety cue (CS-).

Generalization of fear is a learning mechanism whereby fear responses to a CS extend to a range of stimuli (generalization stimuli, or GSs) that resemble the original CS (Pavlov, 1927; Pearce, 1987; Rescorla and Furrow, 1977; Watson and Rayner, 1920). It allows an organism to extrapolate from one experience to other similar events without the necessity to experience them and as such, fear generalization can provide an adaptive advantage. However, overgeneralization

of fear to non-threatening situations can be maladaptive and through excessive proliferation of cues that trigger anticipatory anxiety, fear generalization is considered a critical mechanism in the evolution from an initial panic attack to panic disorder (American Psychiatric Association, 2000). Although described extensively in the older conditioning literature (see Ghirlanda & Enquist, 2003, for a review), human fear generalization has regained interest only lately. To demonstrate generalization of conditioned fear, Lissek et al. (2008) used a differential conditioning procedure in which an electric shock was used as US, a large/small circle as CS+, a small/large circle as CS- and eight intermediate sized circles as generalization stimuli. The strongest fear responses were observed to those circles that were perceptually most similar to the CS+ and fear responding decreased systematically with larger differences from CS+ (Lissek et al., 2008). The continuous downward slope of fear responding to stimuli with gradually less perceptual similarity to the CS+ is called the generalization gradient (Pavlov, 1927) and the strength of fear generalization is indexed by the steepness of the gradient whereby steeper gradients reflect less fear generalization (Lissek et al., 2014).

Because fear generalization seems to depend on the perceptual resemblance of the generalization stimulus to the CS+, the ability to discriminate between stimuli is a relevant factor. Interestingly, several findings have documented perceptual changes concomitant with fear learning (see Zaman et al., 2015 for an overview). For example, fear learning enhances the olfactory system's sensitivity in both animals (Kass, Rosenthal, Pottackal, & McGann, 2013) and humans (Åhs, Miller, Gordon, & Lundström, 2013; Li, Howard, Parrish, & Gottfried, 2008). Resnik, Sobel and Paz (2011) on the other hand demonstrated that aversive conditioning decreased (auditory) discrimination for tones around the CS+, whereas perceptual discrimination for tones around the CS- was improved. Moreover, the physical 'CS+/CS-' distance in auditory

fear conditioning importantly moderates the effect, resulting in (a) increased discrimination when both CSs closely resemble and (b) decreased discrimination with increasing physical distance between CSs (Aizenberg & Geffen, 2013). Together these findings suggest that modulation of perceptual discrimination may be importantly connected with stimulus generalization.

Overgeneralization of conditioned fear to exteroceptive, visual stimuli has been identified as a pathogenic marker of panic (Lissek et al., 2010) and generalized anxiety disorder (Lissek, 2012: Lissek et al., 2014). In particular for panic patients, Lissek et al. (2010) showed a proclivity toward overgeneralization of conditioned fear and the authors suggest that the fear system of panic patients, compared to healthy individuals, is more easily triggered by less robust threat information. The model as used by Lissek et al. (2010) is very well suited to investigate exteroceptive (e.g. agoraphobic) components of panic disorder, but may not necessarily allow firm conclusions on for instance fear responses to somatic arousal that are elicited by fear itself (fear-of-fear). Interoceptive sensations have been hypothesized to play an important role in panic disorder (Barlow, 2002; Bouton et al., 2001), but studies investigating fear generalization to interoceptive fear conditioning (IFC) remain very scarce. Therefore, the present study aimed to investigate fear learning and generalization with interoceptive stimuli, and to explore changes in perceptual discrimination of these interoceptive stimuli in relation to fear generalization.

In contrast to exteroceptive stimuli, interoceptive stimuli are only privately perceived and are often characterized by a vague/systemic location and blurred on- and offset boundaries. Given their perceptually ambiguous nature, strong generalization effects may easily arise with interoceptive stimuli. However, despite its clinical relevance, generalization of interoceptive fear conditioning has not been studied. Fear conditioning paradigms typically apply exteroceptive (e.g., auditory, visual) stimuli as CSs and electro-cutaneous stimuli as US, but many clinical

situations are characterized by internal (interoceptive) rather than external events: cardio-respiratory sensations in panic disorder, proprioception in patients with chronic musculo-skeletal pain, visceral pain and discomfort in functional dyspepsia or irritable bowel syndrome, or increased work of breathing in pulmonary diseases. Therefore, several behavioral theories suggest a critical role of interoceptive fear conditioning (IFC) in the pathogenesis of panic and anxiety disorders (Barlow, 2002; Bouton et al., 2001; Craske & Barlow, 2007; Domschke, Stevens, Pfleiderer, & Gerlach, 2010). Through IFC, initial precursors of a panic attack (US) (minor breathing discomfort, sweating, palpations, heart pounding) become conditioned stimuli (CS), predicting more intense arousal and provoking anxiety as a conditioned response (CR) (Razran, 1961). This anxiety response may produce additional and more interoceptive stimuli (more palpitations, sweating, faster breathing) that further trigger and potentiate fear. A recent experimental paradigm showed successful interoceptive fear conditioning using a non-aversive flow resistor (load) of 10 $cmH_20/l/s$ as CS and a breathing obstruction (occlusion) as US in a paired/unpaired between subject design (Pappens, Smets, Vansteenwegen, Van den Bergh, & Van Diest, 2012). Findings showed that with this ecologically valid CS and panic-relevant US, fear can easily be learned to an initially benign respiratory sensation when this sensation predicts a suffocation experience.

The present study aimed to evaluate the use of another type of panic-relevant, respiratory loads in a modified version of the generalization paradigm as used by Lissek and colleagues (2010). As such, this study aimed to: (a) investigate differential fear conditioning to minor breathing impairments as CSs (inspiratory pressure threshold loads) and a full breathing occlusion as US, (b) assess the degree to which acquired interoceptive fear generalizes to breathing impairments with intensities in-between those of the CS- and CS+, (c) explore

perceptual discrimination between the different breathing obstructions both before acquisition and after generalization and (d) assess pressure threshold loads as conditioned stimuli, instead of flow resistive loads as used by Pappens et al. (2012). In line with the exteroceptive fear generalization paradigm of Lissek et al. (2008), we hypothesized that healthy participants would show successful discriminatory fear learning (conditioned fear responses to the CS+ and not to CS-), and that a generalization gradient would occur, showing consistently less fear responding with decreasing perceptual similarity to the CS+ on the intensity dimension. Furthermore, we hypothesized that enhanced discriminatory learning would be associated with steeper generalization gradients and thus less fear generalization.

Methods and instruments

Participants

Healthy participants were recruited from the student population (N = 43, 29 women; M = 18.63 years, range 18-20). Participants were invited through an online recruitment platform and flyers at the university. In return for participation, they received either two course credits or a financial reward of € 20. All participants provided informed consent and the experiment was approved by the Ethics Committees of the Faculty of Psychology and Educational Sciences as well as the Faculty of Medicine.

Materials and measures

Participants breathed through a breathing circuit that, starting from the participant's side, consisted of a mouthpiece, a bacterial filter, a non-rebreathing valve, and two vinyl tubes of 3.5 x

100 cm (one at either the inspiratory and expiratory port of the non-rebreathing valve). A 3-way stopcock valve at the other side of the inspiratory tube enabled easy switching between loaded breathing (CSs and generalization stimuli (GSs)), the breathing occlusion (US) and unloaded breathing. In contrast to the flow resistive loads as used by Pappens et al. (2012), the present study assessed pressure threshold loads as conditioned stimuli. Threshold positive expiratory pressure (PEP) and inspiratory muscle trainer (IMT) devices (HealthScan Products, Inc; Cedar Grove, NJ) were used as flow-independent inspiratory pressure threshold loads. Both devices feature adjustable specific pressure settings and are commercially marketed to enhance mucus clearance and to reinforce inspiratory muscles. Threshold PEP was used as an inspiratory pressure threshold load by having participants inhale through the exhalation orifice. The PEP is adjustable per 1 cmH_20 within a range 4-20 cmH_20 whereas the IMT is adjustable per 2 cmH_20 within a range 7-41 cmH_20 and in both devices, the pressure-threshold setting is provided by an adjustable, spring-loaded, threshold poppet valve. To inhale, participants have to generate an inspiratory pressure greater than the indicated threshold pressure setting to compress the spring and open the poppet valve. Moreover, the inspiratory pressure must be maintained above threshold pressure to keep the poppet valve open. For participants this feels like a continuous inspiration against a force. If insufficient inspiratory pressure is applied, inspiration is suddenly and completely interrupted. When participants are only on the brink of applying sufficient pressure, this can result in a fluttering sensation in which the valve quickly opens and closes. In order to prevent this from happening, the experimenter instructed the participants in two practice trials to overcome the load pressure. During these trials and the remainder of the experiment, a visually presented timer counting from 8 to 0 informed the participants about the presence and duration of the load, ensuring that they knew the load could and was supposed to be overcome by generating sufficient inspiratory pressure.

Compared to flow resistive loads, pressure threshold loads are less ecologically valid (e.g. no simulation of internal resistance in asthma patients) but their high cost-efficiency and ease of use make them interesting stimuli. Two inspiratory pressure threshold loads (CS1, 6 cmH_20 and CS2, 25 cmH_20) served as conditional stimuli (CSs) and four additional loads of different intensities in between CS1 and CS2 (8, 10.5, 14 and 18.5 cmH_20) were used as generalization stimuli (GSs); loads (CSs and GSs) were always applied for eight seconds. The experimenter applied the load to the inspiratory side of the non-rebreathing valve during the expiratory phase. As such, the actual load-onset co-occurred with the initiation of the subsequent inspiration. The load was applied for 8 s, resulting in at least one loaded inspiratory effort. As unconditioned stimulus (US), an inspiratory breathing obstruction (occlusion) was applied for 40% of the individual's maximal breath holding time (BHT) after full expiration, as determined prior to the conditioning procedure. However, to ensure a significant US, the minimum duration of the US was set at eight seconds, irrespective of the individual's BHT. The real time breathing pattern was used again to ensure that the occlusion was applied before and not during an inspiratory effort. Respiration, electrodermal activity, startle blink responses and subjective US-expectancy ratings were measured.

In order to trigger the startle response, we binaurally administered an acoustic startle probe (102 dB, 50ms with near instantaneous rise time) through headphones. The startle response was measured by recording surface EMG activity over the m. orbicularis oculi. Two Ag/AgCl Sensormedics electrodes (4mm diameter) were placed just beneath the left eye and one reference electrode (4mm diameter) was placed on the forehead. Raw signals were amplified (amplifier coupling: 1Hz) by a Coulbourn isolated bioamplifier with bandpass filter (v75-04; 13Hz-500Hz) and routed to a Coulbourn contour-following integrator (S76-01) that rectified and smoothed the

signal (time constant = 50ms). The signal was then transmitted to a 16-Bit National Instruments PCI-6221 data acquisition card (National Instruments, Austin, Texas) and a personal computer (Dell Optiplex 755; Dell Inc., Round Rock, TX, USA). The EMG signal was recorded from 500 ms before the onset of the white noise probe until 1000 ms after the probe onset. The EMG signal was sampled at and stored at 1000Hz.

Electrodermal activity (EDA) was measured with Fukuda standard Ag/AgCl electrodes (8mm diameter), filled with KY gel and attached to the hypothenar palm of the non-dominant hand. Before attachment, the hand was cleaned with tab water. The interelectrode distance was approximately 2,5 cm. The Coulbourn skin conductance coupler (V71-23) provided a constant 0.5V across the electrodes and the analog signal was passed through a 12-bit A/D converter and digitized at 10Hz.

Throughout the experiment, participants continuously rated their US-expectancy with a custom-built dial (Vansteenwegen, Iberico, Vervliet, Marescau, & Hermans, 2008). Participants were asked to rate their expectancy on a scale that ranged from 0 (certain that breathing occlusion won't occur right now) to 100 (certain that breathing occlusion will occur right now). Similar to EDA, the analog signal was digitized and stored at 10Hz. All signals were sampled and stored with Affect 4.0 software (Spruyt, Clarysse, Vansteenwegen, Baeyens, & Hermans, 2010).

Questionnaires

Before the experimental procedure started, participants filled out the state version of the State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, & Lushene, 1970; Dutch Version: Van der Ploeg, Defares, & Spielberger, 1980). At the end of the experimental procedure, participants were asked to report on their experience during the breathing occlusion and the

pressure threshold load that was used as CS+. For both interoceptive sensations, they were asked to fill in a questionnaire that assessed the extent to which they experienced cued anxiety and fear responses. Therefore, in accordance with the situation-response questionnaire (SRQ) (Pappens et al., 2013), 22 responses were rated on a 5-point Likert scale, ranging from 1 (not at all experienced) to 5 (very strongly experienced). The SRQ items were included for explorative reasons and will not be further discussed as they go beyond the scope of the present manuscript. Also, a 5-point language-free Self-Assessment Manikin (SAM-scale, Bradley and Lang, 1994) was used to retrospectively rate valence, arousal and the experienced degree of control for both the pressure threshold load and occlusion. The valence scale measured how pleasant (1) or unpleasant (5) participants felt during the breathing load and occlusion. The arousal scale measured how calm (1) or excited (5) participants felt during both sensations and the dominance scale assessed whether participants felt no control (1) or full control (5) over the breathing load and occlusion.

Procedure

1. Interoceptive fear conditioning (IFC, Day 1):

The informed consent explained that during the experiment, physiological responses would be measured during different types of stimulation (normal breathing, loaded breathing and complete inspiratory occlusion). After signing the informed consent, participants filled out the STAI-state questionnaire. Next, the experimenter attached the EMG (startle) and EDA (skin conductance) electrodes and assessed the participant's maximal postexpiratory BHT by instructing the participant to hold his/her breath as long as possible following a normal

expiration. Participants raised their hand at the start and end of the breath-holding period and the experimenter started and stopped the stopwatch accordingly. The experimenter explained how to use the breathing circuit and that brief bursts of noise (to record startle EMG) would occur that could be ignored throughout the experiment. Participants were then instructed to continuously rate the degree to which they expected the occlusion to occur. For this purpose a custom made dial was used with a contiunous, 180° radial scale, ranging from 0 (certainly no breathing occlusion) to 100 (certainly a breathing occlusion). Finally, participants were explained that a discrimination test would take place before the actual experiment, allowing them also to become fully acquainted with sensations of respiratory loading. All loads were chosen well above detection threshold (Petersen & Ritz, 2011) and within a range that is not highly aversive and easy to overcome by all participants. Furthermore, participants were informed that they could overcome the load without any problem (that is, open the spring valve by breathing a bit harder) and they were instructed to do so throughout the entire experiment. Adding to this we also provided on screen information indicating “start breathing resistance” at load-onset with a timer counting down from eight seconds, indicating load-presence, till “end of breathing resistance” at load-offset. During the US (occlusion) no such information was given (as occlusions could not be overcome). Prior to the discrimination task, participants received two practice trails during which they practiced overcoming the load each time a visually presented timer indicated the load’s presence and duration. This visual information was presented for every load throughout all phases of the experiment, including pre-exposure, acquisition and generalization.

Participants then took in the mouthpiece, put on the noseclip and started with the discrimination test. In this test, we presented 11 pairs of inspiratory resistive loads (6 loads, 6-8-10.5-14-18.5-25 cmH_20) in which the second load was either identical to or one intensity higher

than the first one. These pairs were presented in a fixed order of an identical pair, followed by an upward comparison, followed by an identical pair, etc. (6-6; 6-8; 8-8; … ; 18.5-25; 25-25). Each load was presented for eight seconds and was indicated on the screen with a label “load 1” or “load 2”. For each pair, participants were asked to rate whether they perceived both loads as (rather) the same or (rather) different. To this end, they used a 0-100 scale, with 50 corresponding to "I don't know"; ratings below 50 respresenting increasing confidence that the loads were equal, and ratings above 50 representing increasing levels of confidence that the loads differed in intensity. The starting position on the scale was 50 for each pair that participants had to rate.

Following completion of the discrimination task, participants received 12 acoustic startle probes (10s between probes) in order to habituate to this acoustic stimulus. Then, participants went through a pre-exposure (3 CS+ and 3 CS- trials in a semi-randomized order) and an acquisition (8 CS+ and 8 CS- trials in a semi-randomized order) phase. Pre-exposure trials consisted of baseline (8s), presentation of the CS+ or CS- (8s) and a subsequent post stimulus interval of 19s without load-presentation. During acquisition, the CS+ was followed by the post-expiratory US in seven out of eight trials (partial reinforcement), whereas the CS- was never followed by the US. For the CS+, acquisition trials consisted of 15s baseline, CS (8s), US (occlusion for 8s or 40% of BHT) and a post stimulus interval (30s). During CS- trials and the unreinforced CS+ trials, the exact same trial sequence and duration were used, but the US was never actually applied. Three seconds before the end of the post stimulus interval, participants were instructed to position the dial back at starting point 0 (certainly no breathing occlusion) at the complete left of the scale. Load intensity was counterbalanced (for half of the participants: CS+ = 6 cmH_20 and CS- = 25 cmH_20). Startle probes occurred four seconds after CS onset, and 21s after the US-offset (in the post stimulus interval). The trial order was fixed and the same CS

was never applied more than three consecutive trials. Table 1 contains a summary of the 'Day 1' IFC-protocol.

Table 1

Summary IFC-Protocol

Discrimination	Habituation	Pre-exposure		Acquisition	
Pairs	Probes	CS (+)	CS (-)	CS+	CS-
11	12	3	3	8	8

2. Generalization (Day 2)

24 hours after fear acquisition, participants returned to the lab for a generalization test. During this test four additional load intensities were presented as generalization stimuli (GSs, 8-10.5-14-18.5 cmH_20) in addition to the CSs (extreme ends of the range, 6 and 25 cmH_20). Upon arrival, participants were seated and the experimenter attached EMG and EDA electrodes. Participants were then reminded on how to use the breathing circuit and informed that again brief bursts of noise would occur that could be ignored throughout the experiment. Participants were re-instructed to continuously rate the degree to which they expected the occlusion to occur. Participants received first 12 acoustic startle probes (10s between probes) in order to habituate to this stimulus. Then, the generalization test started during which the CS+ remained reinforced whereas neither the GSs, nor the CS- was followed by a breathing occlusion. The CS+ remained reinforced in order to prevent fear extinction, which would compromise generalization of conditioned fear. All stimuli were presented twice in a fixed order with the restriction that each stimulus occurred only once each three consecutive trials. Trials had the same time structure and

measurements as during acquisition (previous day). Also similar to acquisition, visual on screen information indicated the loads' presence. Following this generalization phase, participants were explained that a discrimination test would take place again to conclude the experiment and that the procedure was similar to day 1. After the discrimination test and before debriefing, a subsample (N=18) was asked to estimate the number of different load intensities used. Out of this subsample, 50% indicated to having experienced five or more load intensities, whereas another 50% indicated about three to four loads.

Data reduction and analysis

Data reduction and parameter extraction

Expectancy

As we were primarily interested in how participants' US expectancy changed when CSs or GSs were administered, we subtracted the US-expectancy of the first second of the CS/GS from the US-expectancy from the last second during which the CS/GS was on (see also Pappens et al., 2012). Thus, a positive change score means that the CS/GS caused participants to increase their expectancy of the US, whereas a negative change score means that the CS/GS was associated with a decrease in US-expectancy. These change scores were averaged across the three pre-exposure trials (Pre-exp Block), across trials 1-4 of acquisition (Acq1 Block), across trials 4-8 of acquisition (Acq2 Block) and across trials 1-2 per CS and GS in the generalization phase. Seven participants out of 43 were excluded from US-expectancy analysis due to technical failures or obvious misinterpretation of the instructions. Thus, data of 36 participants were used for analyses (Cond_LowCS+: N = 16, Cond_HighCS+: N = 20). From the dataset on perceptual

discrimination, three additional participants were excluded due to misinterpretation of the instructions (N = 33, Cond_LowCS+ = 14 and Cond_HighCS+ = 19).

Startle Eyeblink responses

Startle eyeblink responses were calculated by subtracting the mean value for the baseline 0-20 ms time window following probe onset from the peak value detected in the 21-175 ms time window after probe onset . All individual raw startle responses were visually checked and labeled as (1) 'non response' if no response could be visually identified in the 21-175ms time window as compared to baseline activity or (2) 'reject' if excessive baseline activity or movement artifacts were present. If at least 30% of all startle responses were rejected (28 out of 92 trials), participant's data were excluded from further analyses. Also, only data of participants who show startle blink responses in more than 30% of trials were included. As such, out of 43 participants, two were excluded due to technical failure and excessive baseline activity and 27 were labeled as "non-responders". Inter-rater reliability on the labeling procedure reached 95% on a subsample of three participants, hereby confirming (a) the applied labeling criterion and (b) the high number of 'non responses' present in the data. When, as inclusion criteria, at least four valid cases (= not labeled as 'non response', nor 'rejected') out of eight trials are requested per cell in the acquisition data matrix, only nine persons out of 41 could be included for analysis. Similarly, when at least two valid cases are required per cell in the generalization data matrix, only eight participants could be included in further analysis and out of these eight, only three were included in acquisition analysis as well. As this N is too small to allow for meaningful statistical analyses, we decided to not statistically analyze the startle data.

Skin conductance response

Electrodermal responses (skin conductance response, SCR) were calculated by subtracting the mean skin conductance level during two seconds prior to CS/GS onset from the maximum SCL during six seconds following CS/GS onset. Responses were averaged across the three pre-exposure trials (Pre-exp Block), across trials 1-4 of acquisition (Acq1 Block), across trials 4-8 of acquisition (Acq2 Block) and across trials 1-2 per CS and GS in the generalization phase. SCR-data were then log transformed [LOG10(SCR+1)]. We did focus on the 0-6s CS period in order to make sure that no acoustic startle probe was included. Due to technical failure, data of two out of 43 participants were excluded (N = 41, Cond_LowCS+ = 20 and Cond_HighCS+ = 21).

Respiratory responses

Inspiratory time (Ti, in sec), expiratory time (Te, in sec), tidal volume (Vt, in ml),and mean inspiratory flow (Vt/Ti in ml/s) were calculated for all loaded breaths in the CS-interval as well as unloaded breaths during baseline. Responses were averaged for each trial and final parameters were calculated by subtracting the mean value during CS from the baseline value. These difference scores were then averaged across three pre-exposure trials (Pre-exp Block), across trials 1-4 of acquisition (Acq1 Block), across trials 4-8 of acquisition (Acq2 Block) and across trials 1-2 per CS and GS in the generalization phase. Due to technical failure, data of three out of 43 were excluded on day 1 (N = 40, Cond_LowCS+ = 20 and Cond_HighCS+ = 20) as well as three on day 2 (N = 40, Cond_LowCS+ = 19 and Cond_HighCS+ = 21).

Data analysis

All analyses were performed with repeated measures ANOVAs (RM ANOVA), using a rejection criterion of $p < .05$. Data of day 1 and 2 were analyzed separately. For pre-exposure and acquisition (day 1), within-subject variables included 'CS' (+/-) and 'Block' (Pre-exp, Acq1, Acq2), whereas 'Condition' was a between-subject variable (Cond_LowCS+, CS+ = 6 cmH_20; Cond_HighCS+, CS+ = 25 cmH_20). Data of the Generalization phase (day 2) were analyzed in a separate RM ANOVA, with 'Load' (CS1-G1-G2-G3-G4-CS2) as within and 'Condition' (Cond_LowCS+/Cond_HighCS+) as a between-subject variable. Perceptual discrimination was explored with 'Time' (prior to acquisition; after generalization), 'Type' (different versus identical load pair) and 'Intensity' (five comparisons) as within-subject variables; 'Condition' was included as between-subject variable with two levels. Respiratory data were analyzed separately for Pre-exposure and Acquisition on day 1. Greenhouse-Geisser corrections were applied where appropriate; uncorrected degrees of freedom and corrected p-values will be reported together with η_p^2.

To explore to what extent differential learning during acquisition related to generalization, we calculated an index of differential fear learning in US-expectancy for each participant. This 'learning index' reflects the change in the differential effect (CS+ minus CS-) from pre-exposure to the last block of acquisition. The underlying idea of this index was that differential learning in participants is present only when the CS+/CS- difference at the end of acquisition exceeds a CS+/CS- difference prior to acquisition (pre-exposure). Specifically for generalization, a multiple regression for each subject was conducted with the rank order of the load intensity as predictor for the US-expectancy change scores. The resulting beta-coefficients indicate the individual generalization slopes along the load intensity gradient. Negative coefficients indicate that US-expectancy decreased with increasing load intensities, a pattern that could be expected for

participants from Cond_LowCS+ who received the lowest load intensity (CS1) as the CS+ and the highest intensity (CS2) as the CS-. Conversely, positive coefficients indicate that US-expectancy increased with increasing load intensity, a pattern that can be expected for Cond_HighCS+ where the highest load (CS2) served as the CS+ and the lowest intensity (CS1) as the CS-. To investigate to what extent differential learning during acquisition (day 1) related to generalization gradients on day 2, we calculated for each condition a Pearson product-moment correlation between the above mentioned 'learning index' and the Fisher z-transformed beta-coefficients reflecting generalization gradients.

Results

Breath holding time and questionnaires

A near significant difference was found in mean BHT between Cond_LowCS+ (CS+ = 6 cmH_20; M = 25.75s, SD = 10.50s) and Cond_HighCS+ (CS+ = 25 cmH_20; M = 20.30s, SD = 6.20s), $t(34) = 1.94$, $p = .06$. No difference was found in mean STAI-state results between both groups, Cond_LowCS+ (M = 36.44, SD = 7.97) and Cond_HighCS+ (M = 36.45, SD = 6.15), $t(34) = .005$, $p = .996$.

US-expectancy

1. Pre-exposure and acquisition:

Participants increased their US-expectancy more for CS+ than for CS- (main effect of 'CS', $F(1, 34) = 14.54$, $p < .001$, $\eta_p^2 = .30$). US-expectancy ratings were higher during Acquisition compared to Pre-exposure (main effect for 'Block', $F(2, 68) = 5.32$, $p < .05$, $\eta_p^2 =$

.14, ε = .82). Overall, differential learning effects occurred by the end of Acquisition (2-way 'CS' * 'Block' interaction, $F(2,68) = 9.60$, $p < .001$, $\eta_p^2 = .22$, ε = .80), but were highly affected by the stimulus intensity that was used as CS+/CS- (3-way 'CS' * 'Block' * 'Condition' interaction, $F(2, 68) = 8.58$, $p < .001$, $\eta_p^2 = .20$, ε = .80). Figure 1 displays the latter 3-way interaction and suggests that differential learning was established only in the group who received the load of 6 cmH_2O as CS+ (Cond_LowCS+). Overall raw expectancy ratings remained well within the scale boundaries and ranged between 35 and 74 on the scale of 0-100 so no floor or ceiling effects were observed with regard to the rating scale. Figure 2 displays a mean of the absolute ratings for each block and both conditions 'Cond_LowCS+ and 'Cond_HighCS+'.

Four follow-up comparisons were used to further investigate differential learning effects using a rejection criterion of $p < .0125$ (Bonferroni correction for multiple comparisons). These follow-up comparisons confirmed that in Cond_LowCS+ (CS+ = 6 cmH_20), US expectancy in the last acquisition block increased more to the CS+ relative to the CS-, ($F(1,34) = 23.95$, $p < .001$), whereas such differential effect was absent during pre-exposure ($F(1.34) = 4.43$, $p = .04$). Participants who received the load of 25 cmH_2O as the CS+ (Cond_HighCS+) tended to increase their US expectancy more during CS+ than during CS- in the last acquisition block ($F(1,34) = 4.30$, $p = .05$), but this effect does not reflect learning as it was already present during pre-exposure ($F(1,34) = 7.93$, $p < .01$). Furthermore, participants scoring higher on STAI-state showed a smaller differential 'learning index' at the end of acquisition ($r(36) = -.36$, $p < .05$).

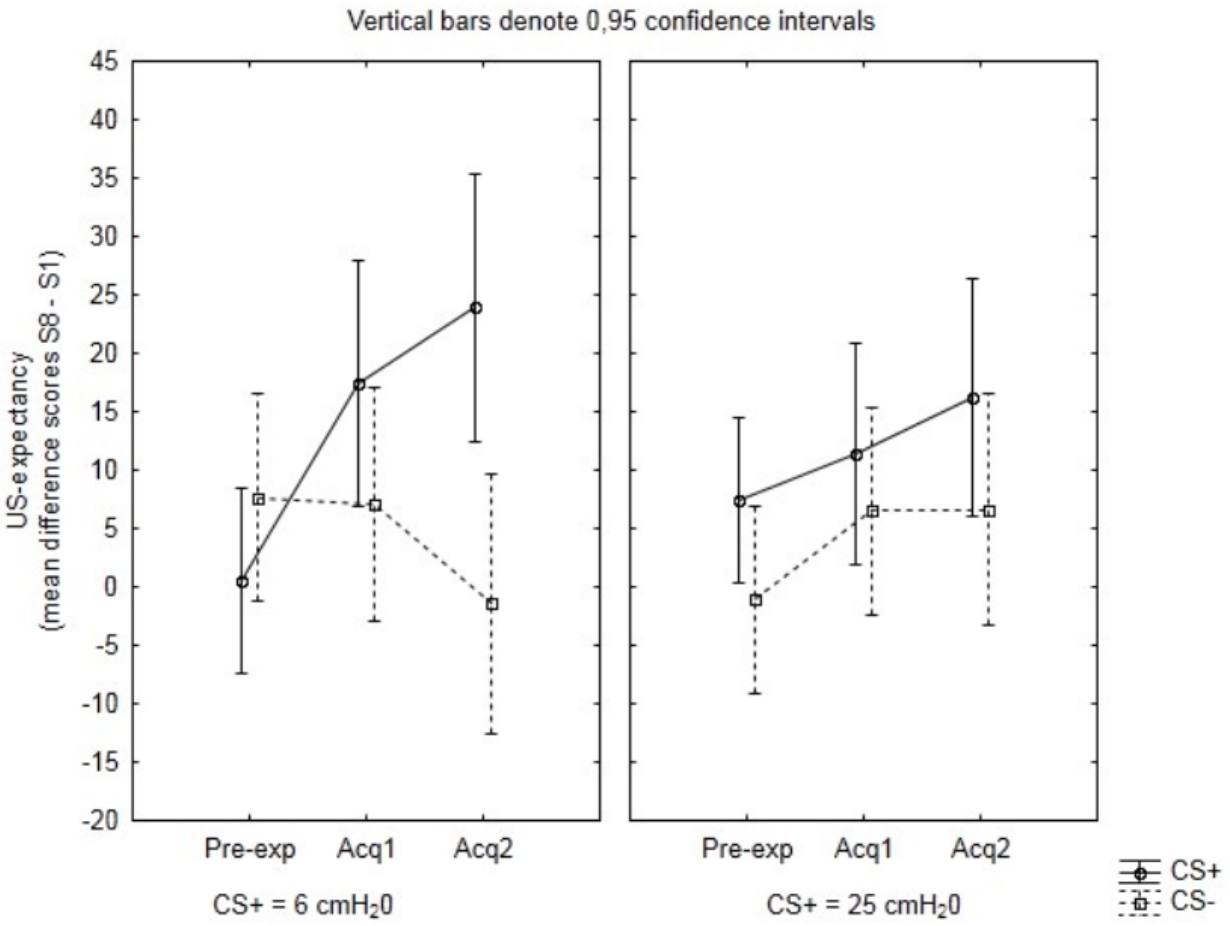

Figure 1: Change in US-expectancy during the CSs per Block (Pre-exp, Acq1 and Acq2). At the end of acquisition, differential learning is installed in Cond_LowCS+ (CS+ = 6 cmH_20) but impaired in Cond_HighCS+ (CS+ = 25 cmH_20).

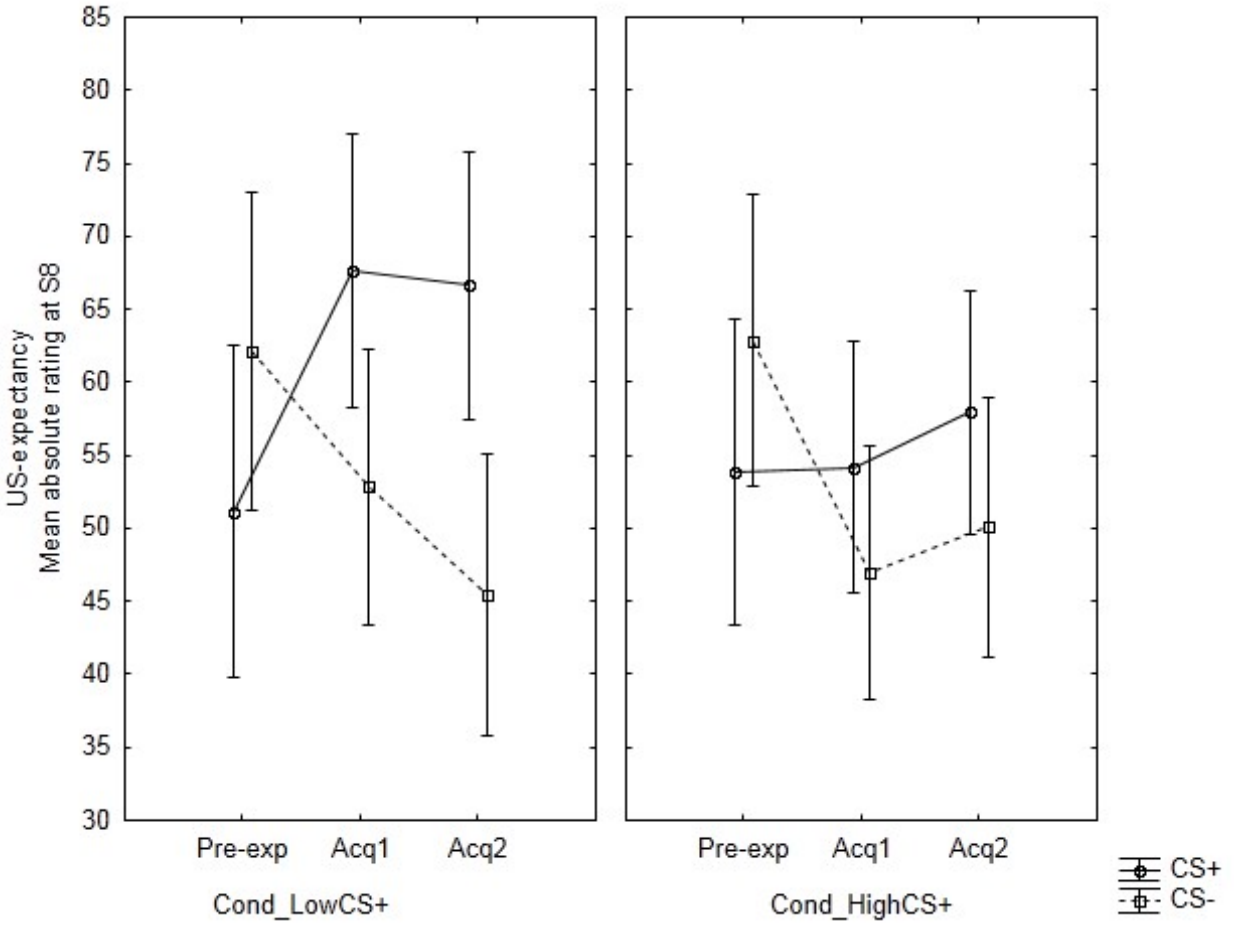

Figure 2: Mean absolute values of US-expectancy (0-100 scale) during the last second of CS-presentation (S8) on the rating scale 0-100.

2. Generalization:

As shown in Figure 3, the US-expectancy of participants in Cond_LowCS+ (CS+ = 6 cmH_20) changed in function of load intensity, whereas participants in Cond_HighCS+ (CS+ = 25 cmH_20) responded in a similar way to all load intensities and thus seem to generalize their fear response to all load intensities (2-way 'Load' * 'Condition' interaction, $F(5,170) = 6.44$, $p <$

.001, η_p^2 = .16 , ε = .86; main effect of 'Condition', $F(1, 34) = 5.58$, $p < .05$, η_p^2 = .14; main effect of 'Load', $F(5, 170) = 3.19$, $p < .05$, η_p^2 = .09, ε = .86).

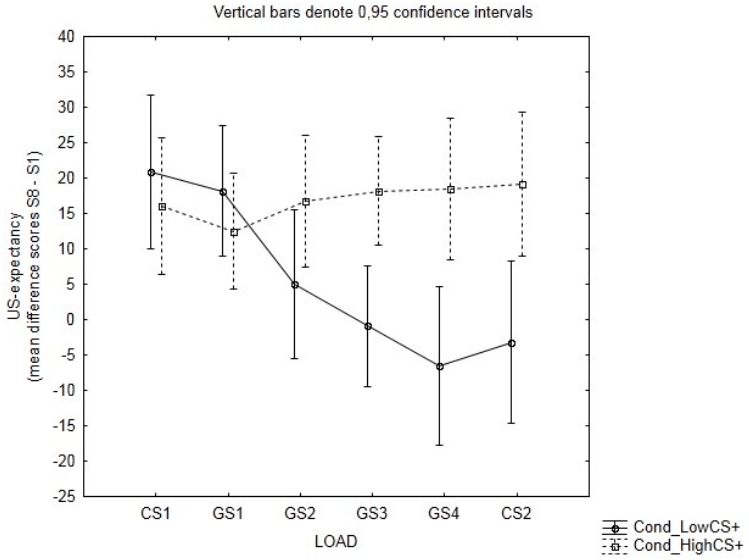

Figure 3: Change score in US-expectancy during presentation of the CSs and GSs (averaged across both generalization trials). Cond_LowCS+ shows a differentiated fear response along the continuum of load intensities (= generalization gradient) whereas Cond_HighCS+ shows no such gradient (= complete generalization).

To identify the point on the continuum of load intensity at which US-expectancy ceased to generalize, post hoc follow up comparisons were conducted in which expectancy ratings towards the conditioned safety cue were compared against the conditioned danger cue as well as all intermediate load intensities (Bonferroni corrected rejection criterion for 10 comparisons $p < .005$). For Cond_LowCS+, US expectancy responses towards CS1 (= CS+) are increased ($F(1,34) = 15.70$, $p < .001$) and generalize towards GS1 ($F(1,34) = 12.59$, $p < .005$) but not to GS2 ($F(1,34) = 1.46$, $p = .23$), GS3 ($F(1,34) = .24$, $p = .63$) and GS4 ($F(1,34) = .42$, $p = .52$). In

contrast, for Cond_HighCS+, US expectancy responses towards CS2 (= CS+) are not increased ($F(1,34) = .33$, $p = .57$) and neither are responses towards GS4 ($F(1,34) = .18$, $p = .67$), GS3 ($F(1,34) = .14$, $p = .71$), GS2 ($F(1,34) = .02$, $p = .88$) and GS1 ($F(1,34) = .87$, $p = .36$). However, the overall US-expectancy is elevated in Cond_HighCS+ indicating equal fear responding to all load intensities.

To further explore a potential association between generalization (day 2) and differential learning (day 1), multiple regressions were run for each participant with the load intensity rank order as predictor for the US-expectancy change scores during the generalization phase. The resulting beta-coefficients are thus indicative for the increase or decrease in US-expectancy according to the load intensities. Subsequently, these coefficients were Fisher z-transformed before entering them in a Pearson product-moment correlation with the 'learning index'. For Cond_LowCS+, a significant negative correlation was shown ($r(16) = -.59$, $p < .05$) and a positive, non-significant, correlation for Cond_HighCS+ ($r(20) = .39$, $p = .09$). These data show that in both conditions, stronger differential learning coincides with steeper generalization gradients.

Respiratory behavior

Tables 2 and 3 display the mean changes (and SD) in Ti, Te, tidal volume and mean inspiratory flow from baseline to the load for day 1 (Pre-exposure and Acquistion) and day 2 (Generalization), respectively.

1. Pre-exposure and acquisition:

During pre-exposure, the stronger load (25 cmH_20) caused more pronounced increases in inspiratory and decreased expiratory time as compared to the lighter load (6 cmH_20), 'CS' * 'Condition' interaction for Ti, $F(1,38) = 8.38$, $p < .01$, $\eta_p^2 = .18$ and Te, $F(1,37) = 12.07$, $p < .005$, $\eta_p^2 = .25$, respectively. These stimulus intensity effects for Ti and Te persisted throughout acquisition, 'CS' * 'Condition' interactions for Ti, $F(1,37) = 6.63$, $p < .05$, $\eta_p^2 = .15$; and Te, $F(1,37) = 24.09$, $p < .001$, $\eta_p^2 = .39$. No learning effects were present for Ti and Te (non-significant 3-way 'CS' * 'Block' * 'Condition' interactions for Ti, $F(1,37) = .54$, $p = .47$, $\eta_p^2 = .01$; and for Te, $F(1,37) = .77$, $p = .39$, $\eta_p^2 = .02$.

Tidal volume decreased more in response to the stronger as compared to the lighter load, both during pre-exposure ('CS' * 'Condition', $F(1,37) = 19.23$, $p < .001$, $\eta_p^2 = .34$) and acquisition ('CS' * 'Condition', $F(1,36) = 55.97$, $p < .001$, $\eta_p^2 = .61$). The 3-way interaction was not significant ('CS' * 'Block' * 'Condition', $F(1,36) = .02$, $p = .88$, $\eta_p^2 = .00$).

Similarly, mean inspiratory flow decreased more in response to the stronger as compared to the lighter load ('CS' * 'Condition' interaction for pre-exposure, $F(1,37) = 36.48$, $p < .001$, $\eta_p^2 = .50$, and for acquisition, $F(1,37) = 82.88$, $p < .001$, $\eta_p^2 = .69$). Again, the 3-way interaction was not significant ('CS' * 'Block' * 'Condition', $F(1,37) = .92$, $p = .34$, $\eta_p^2 = .02$).

Table 2
Mean difference scores per phase for all respiratory parameters

		DAY 1			
Cond_LowCS+		Pre-exp CS+	Pre-exp CS-	AcqCS+	AcqCS-
	Ti	0.23	0.67	0.40	0.64
		(0.52)	(1.05)	(0.39)	(0.68)
	Te	-0.41	-0.63	-0.43	-0.67
		(0.37)	(0.53)	(0.23)	(0.33)
	Vt	-76.61	-301.69	-39.28	-286.87
		(233.60)	(277.66)	(154.68)	(221.02)
	Mean insp flow	-128.94	-321.43	-122.00	-327.79
		(118.47)	(146.30)	(94.28)	(119.48)
Cond_HighCS+		Pre-exp CS+	Pre-exp CS-	AcqCS+	AcqCS-
	Ti	0.86	0.51	0.92	0.64
		(0.93)	(0.43)	(0.88)	(0.59)
	Te	-0.60	-0.27	-0.65	-0.36
		(0.41)	(0.17)	(0.55)	(0.31)
	Vt	-235.09	-44.94	-238.62	18.56
		(316.79)	(182.54)	(250.39)	(222.10)
	Mean insp flow	-276.99	-145.78	-238.88	-111.31
		(81.18)	(96.25)	(93.53)	(122.17)

Note. Ti = inspiratory time (in s); Te = expiratory time (in s);
Vt = tidal volume (in ml) and mean inspiratory flow is Vt/Ti (in ml/s)

2. Generalization

During the generalization phase, the main effects of load intensity were significant for Ti ($F(5,170) = 3.10$, $p < .05$, $\eta_p^2 = .08$); Te ($F(5,185) = 5.85$, $p < .001$, $\eta_p^2 = .14$); tidal volume ($F(5,175) = 21.45$, $p < .001$, $\eta_p^2 = .38$) and mean inspiratory flow ($F(5,185) = 35.70$, $p < .001$, $\eta_p^2 = .49$). As can be read from Table 3, respiratory responses to loads grew stronger with higher intensities of the loads (stronger increases in Ti, stronger decreases in Te, Vt and mean

inspiratory flow), although this seems less the case for the loads with the three highest intensities (G3, G4, CS2), which triggered more similar responses.

Table 3
Mean difference scores per phase for all respiratory paramters

		DAY 2					
Cond_LowCS+		CS1	G1	G2	G3	G4	CS2
	Ti	0.38	0.42	0.52	0.79	0.42	0.62
		(0.71)	(0.71)	(0.67)	(1.10)	(1.00)	(0.64)
	Te	-0.37	-0.61	-0.72	-0.76	-0.84	-0.71
		(0.32)	(0.48)	(0.39)	(0.79)	(0.69)	(0.38)
	Vt	-12.80	-95.89	-251.63	-380.04	-355.59	-324.26
		(276.23)	(290.46)	(253.38)	(361.96)	(312.11)	(249.17)
	Mean insp flow	-77.42	-156.62	-240.51	-318.12	-324.46	-323.04
		(175.61)	(148.85)	(120.77)	(161.40)	(164.19)	(144.87)
Cond_HighCS+		CS1	G1	G2	G3	G4	CS2
	Ti	0.50	0.72	0.92	0.95	0.98	1.00
		(0.53)	(0.76)	(0.85)	(1.03)	(0.93)	(0.97)
	Te	-0.35	-0.60	-0.55	-0.81	-0.66	-0.71
		(0.40)	(0.78)	(0.62)	(0.53)	(0.74)	(0.72)
	Vt	83.14	67.07	- 105.84	-258.47	-98.67	-119.47
		(189.28)	(231.79)	(276.13)	(307.40)	(374.54)	(247.47)
	Mean insp flow	-63.29	-113.19	-198.84	-253.19	-227.25	-236.50
		(111.27)	(153.14)	(158.04)	(157.02)	(150.12)	(144.72)

Note. Ti = inspiratory time (in s); Te = expiratory time (in s);
Vt = tidal volume (in ml) and mean inspiratory flow is Vt/Ti (in ml/s)

Skin conductance response

A significant 'CS' * 'Block' interaction suggested differential effects at the end of acquisition with a reduced response pattern to CS+ and increased response pattern to CS- over time ($F(2,78) = 9.48$, $p < .001$, $\eta_p^2 = .20$, $\varepsilon = .82$), see figure 4. However, one follow-up comparison indicated no significant differential effect at the end of acquisition ($F(1,39) = 3.25.$, $p < .08$).

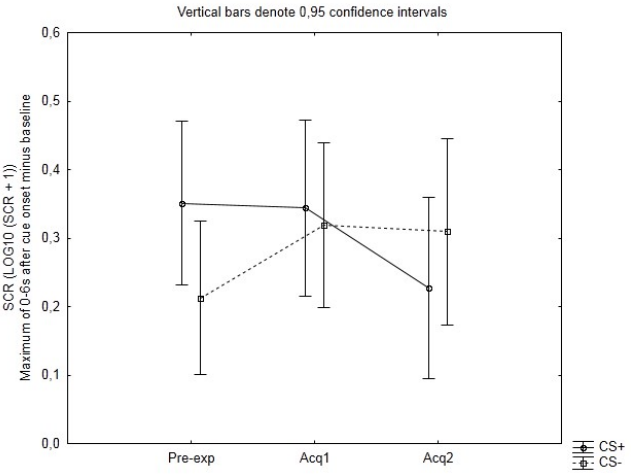

Figure 4: Skin conductance responses (SCR-Log MicroSiemens) during the CSs per Block (Pre-exp, Acq1 and Acq2). No significant differences were observed between both conditions and therefore the figure is not split between 'Cond_LowCS+' and 'Cond_HighCS+'.

Perceptual discrimination

Overall, participants rated identical and different pairs as more identical (< 50) and more different (> 50) from each other, respectively (main effect of 'Type', $F(1,31) = 12.09$, $p < .005$, $\eta_p^2 = .29$). However, different pairs were rated less dissimilar following generalization relative to prior to acquisition, indicating hampered perceptual discrimination after the generalization phase (two way interaction of 'Time' * 'Type', $F(1,31) = 8.89$, $p < .01$, $\eta_p^2 = .22$). Furthermore, perceptual discrimination after generalization tended to be more affected in Cond_LowCS+, compared to Cond_HighCS+ (near significant three way interaction 'Time' * 'Type' * 'Condition', $F(1,31) = 3.84$, $p = .06$, $\eta_p^2 = .11$; as shown in figure 5). Four follow-op contrasts, using a rejection criterion of $p < .0125$ (Bonferroni correction for multiple comparisons), were used to investigate differential responding to same and different pairs. Before acquisition in Cond_LowCS+, different pairs were rated significantly higher (as more different) than similar pairs ($F(1,31) = 29.23$, $p < .001$), but this effect was absent after generalization ($F(1,31) = 0.17$, $p = .69$). In Cond_HighCS+, different pairs were again rated significantly higher than similar pairs ($F(1,31) = 14.32$, $p < .001$), whereas both type of pairs were treated more similar after generalization ($F(1,31) = 1,72$, $p = .20$).

In general, perceptual discrimination was better for loads in the lower intensity range, whereas participants seem to discriminate less between loads above 18,5 cmH_2O (two way interaction 'Type' * 'Intensity', $F(4,124) = 2.90$, $p < .05$, $\eta_p^2 = .09$, $\varepsilon = .81$). Overall, pairs in the lower range were rated as more different before acquisition than after generalization (two way interaction 'Time' * 'Intensity', $F(1,124) = 3.78$, $p < .05$, $\eta_p^2 = .11$, $\varepsilon = .71$) and perceptual discrimination in the lower range was stronger in Cond_HighCS+ (three way interaction 'Type' * 'Intensity' * 'Condition', $F(4,124) = 3.50$, $p < .05$, $\eta_p^2 = .10$, $\varepsilon = .81$).

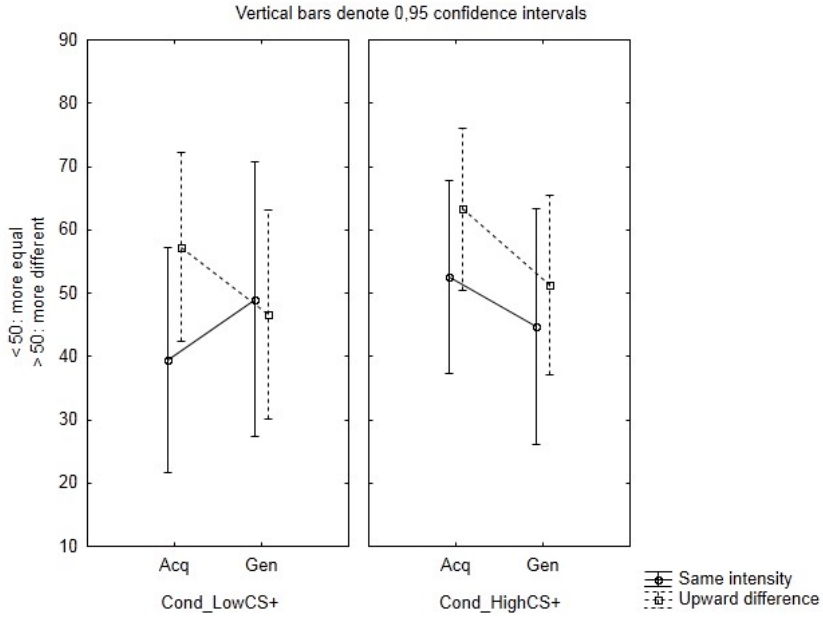

Figure 5: Before acquisition, pairs of different intensity were rated as more different in both conditions. After generalization, identical and different pairs were rated more similarly, indicating a drop in perceptual discrimination after the generalization phase. This drop was clearly present for participants in Cond_LowCS+, compared to Cond_HighCS+.

Questionnaires and self-report

Questionnaire data were correlated with US-expectancy outcomes, showing that participants who report more anxiety before the experiment (STAI-state questionnaire), show less differential fear learning ($r(36) = -.36$, $p < .05$). Furthermore, participants who retrospectively felt more in control over the CS+ pressure threshold load, showed increased differential learning ($r(36) = .41$, $p < .05$) and reported less anxiety prior to the experiment ($r(36) = -.51$, $p < .05$). In

turn, more unpleasantness, experienced during the CS+, correlated with decreased learning effects ($r(36) = -.41$, $p < .05$).

Discussion

The first aim of the current paradigm was to investigate interoceptive fear conditioning and generalization in a differential paradigm that allows to study within-subject fear learning towards CS+, safety learning towards CS- and fear generalization towards intensities that are located in-between CS+ and CS-. The second aim was to explore performance on a perceptual discrimination task prior to acquisition and after generalization. We hypothesized that (a) healthy participants would show successful discriminatory fear learning (stronger conditioned fear responses to the CS+ than to CS-), (b) that fear would generalize stronger to stimuli located closer the CS+, resulting in the observation of a generalization gradient and (c) that enhanced discriminatory learning would be associated with a reduced fear generalization and thus steeper generalization gradients. A final aim was to assess the applicability of pressure threshold loads as conditioned stimuli (CSs).

Our findings show that low load intensities are more likely to enter into CS-US associations than higher load intensities. In the group where the light load was used as CS+, there was no initial differential expectation during pre-exposure after which fear learning can be observed already in the first acquisition block. Safety learning seemed to come about later (second acquisition block), but was overall limited with only small decreases in US expectancy (difference scores just below zero). On the other hand, in the group where the strong load was used as CS+, there was an a priori differential expectation during pre-exposure, which remained throughout acquisition. Learning seems thus modulated by pre-existing expectations regarding

the US-occurrence, based on stimulus intensity and/or CS - US resemblance. This is in line with models posing that associative learning is stronger when the CS is paired with an unexpected US (Bradfield & McNally, 2008; Rescorla & Wagner, 1972). As such, fear conditioning is thought to depend on an 'error' signal reflecting the discrepancy between US expectation and actual US presence (McNally, Johansen, & Blair, 2011). In our first group (Cond_LowCS+) this error signal will be elevated, explaining the fast fear learning curve. Pressure threshold loads remain closed until sufficient inspiratory force is applied to open the valve. Even though this initial occlusion is easy to overcome, some CS properties can intrinsically refer to the US sensation and may as such explain slow safety learning, as observed in Cond_LowCS+. For Cond_HighCS+ on the other hand, the a priori expectation that the stronger CS+ will be followed by the US, is not violated by US-occurrence and very small error signals might thus explain the absence of significant discriminatory fear learning. Intriguing is the apparent increase in US-expectancy during the CS- in early acquisition for the Cond_HighCS+ group, which may indicate a failure or very late onset of safety learning to the low intensity CS-.

Interestingly, the presence of discriminatory learning on day 1 was related to the shape of the generalization gradient on day 2. In Cond_LowCS+, where discriminatory learning was significantly present, US-expectancies for the higher intensity GSs gradually decreased along the stimulus intensity dimension, which may reflect a “generalized safety response” towards these loads and is very much in line with previous findings from exteroceptive fear generalization (Dunsmoor, Mitroff, & Labar, 2009; Ghirlanda & Enquist, 2003). In contrast, when no discriminatory learning was acquired (Cond_HighCS+), all load intensities elicited a consistent increase in US-expectancy, or, fear generalized completely to all stimulus intensities. The association between discriminatory learning and fear generalization was also confirmed by

correlation analyses, showing that stronger differential learning in acquisition was associated with a steeper generalization gradient. In the same vein, work in animals has shown that rats in a single cue paradigm show stronger fear generalization (freezing behavior) than rats in a differential conditioning paradigm (Aizenberg & Geffen, 2013). As such, our findings extend evidence from work in animals by suggesting that safety or discriminatory learning may limit fear generalization in humans. However, the present data do not allow to conclude on whether both phenomena are causally related to each other, as another, yet unkown third group factor (e.g., a pre-existing group differences, salience/intensity of the CS+, CS+ - US resemblance) may influence both safety learning and fear generalization independently from each other. In such case, adding more acquisition trials may facilitate safety learning towards the end of acquisition, while it may not necessarily affect the fear generalization gradient. Even if safety learning and fear generalization would entail a causal relationship, the present data could not reveal its directionality. Indeed, it might also be that safety learning during acquisition did not occur, because of fear generalization to the safety cue (CS-) taking place already early on during acquisition. Thus, the process of fear generalization might act before and compete with actual safety learning.

After generalization, participants showed an overall decreased perceptual discrimination between adjacent stimulus intensities, compared to their discrimination before acquisition. When stimuli are helpful in the prediction of aversive events, organisms are more motivated to perceptually discriminate between stimuli. The observed decrease in discrimination performance might be explained by the fact that the discrimination task in itself, was not relevant for the prediction of US-occurrence in the present experiment. Also, the tendency toward better discrimination in Cond_HighCS+, can be explained by the motivational relevance to

discriminate. This group showed less differential learning and strong fear generalization, compared to Cond_LowCS+; as such all stimulus intensities may retain increased predictive value, making them motivationally more relevant to discriminate.

The lack of evidence for conditioning in electrodermal activity and respiration was unexpected, as previous findings with flow-dependent, inspiratory resistive loads did show successful fear conditioning in both peripheral physiology and respiratory behavior (e.g., Pappens et al., 2012; 2013; 2014; 2015). One speculative explanation for the non-findings in the present study may relate to the use of pressure threshold loads as CSs. The respiratory manoeuvers elicited by this type of load may override the fear responses in electrodermal activity. Likewise, stimulus driven effects of this type of loads may override eventual fear conditioning effects in respiratory behavior. In the present study, we unexpectedly failed to obtain an adequate number of startle responses. Possible explanations include: (1) the respiratory manoeuvers in response to the pressure threshold loads may somehow have affected startle responding, (2) participants' attention may have been directed away from the exteroceptive startle probe, in favor of a greater interoceptive attention or (3) technical anomalies during data acquisition that remained unnoticed at the time of the experiment (e.g., bad electrode surface, missing startle probes due to technical issues).

A potential limitation of the present study is that the findings are only based on US-expectancy measures. US-expectancy is thought to reflect contingency awareness, but not necessarily fear. Nonetheless, danger expectancies are key elements of fear responding and fear learning (Grillon, 2002, 2008; Davey, 1992, 2006). Ohman and Mineka (2001) proposed contingency learning together with emotional learning to constitute two levels of learning that are at play in human fear conditioning. Boddez et al. (2013) recently evaluated US-expectancy as a

measure of conditioned fear learning and argue that US-expectancy may indeed be sensitive to contingency learning but still remains a valid index of fear learning because danger expectancies should be included in the conglomerate of fear responses. As our US was aversive and previous studies with the same type of US (but other CSs), have consistently found fear conditioning in startle responses (Pappens et al., 2012; 2014), it is justified to assume that the US-expectancy measure in the present study does reflect a component of fear responding. Still, a stronger case would have been made if the effects would emerge also in measures that are thought to reflect defensive responding.

Other potential limitations include the fixed durations for baseline, CS and ITI, and the visual information that was used to confirm the presence of the loads. Regarding the latter, an invariant exteroceptive component was added to the interoceptive stimuli that served as CS+, CS- or GSs. From a theoretical viewpoint, the visual cue may have served as an occasion setter, as the US never occurred without being preceded by this visual cue, but the visual cue itself is not a valid predictor the US.

Finally, a clear limitation relates to the discrimination test in which we used consecutive, single presentations of alternating equal and different load pairs in an ascending fixed order. We opted for this test mainly for pragmatic reasons. Methodological improvements of the task would include multiple presentations of each pair in a random order. Another problem with the discrimination task may be that especially on day 2, participants' performance may have been influenced by fatigue or a general lack of motivation.

To summarize, our findings show firstly that differential fear learning with interoceptive stimuli seems modulated by stimulus intensity of the CSs and secondly that the occurrence of

discriminatory learning is closely related to fear generalization. Safety learning might be essential to prevent fear generalization and the current paradigm encourages a stronger focus on the causal mechanisms that underlie safety learning and fear generalization. Thirdly, following generalization, participants showed an overall decreased perceptual discrimination between adjacent stimulus intensities, compared to their discrimination before acquisition.

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